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**A multicellular approach forms a significant amount of tissue-engineered small intestine in the mouse.**

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**Public Summary:**

**Scientific Abstract:**

Tissue-engineered small intestine (TESI) has successfully been used to rescue Lewis rats after massive small bowel resection. In this study, we transitioned the technique to a mouse model, allowing investigation of the processes involved during TESI formation through the transgenic tools available in this species. This is a necessary step towards applying the technique to human therapy. Multicellular organoid units were derived from small intestines of transgenic mice and transplanted within the abdomen on biodegradable polymers. Immunofluorescence staining was used to characterize the cellular processes during TESI formation. We demonstrate the preservation of Lgr5 and DcamK1 positive cells, two putative intestinal stem cell populations, in close proximity to their niche mesenchymal cells, the intestinal subepithelial myofibroblasts (ISEMF) at the time of implantation. Maintenance of the relationship between ISEMF and crypt epithelium is observed during the growth of TESI. The engineered small intestine has an epithelium containing a differentiated epithelium next to an innervated muscularis. Lineage tracing demonstrates that all the essential components, including epithelium, muscularis, nerves, and some of the blood vessels, are of donor origin. This multicellular approach provides the necessary cell population to regenerate large amounts of intestinal tissue that could be used to treat short bowel syndrome.

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